AWARD NUMBER: W81XWH-14-1-0111

TITLE: A Molecular Framework for Understanding DCIS

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REPORT DATE: October 2015

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	30 Sep 2014 - 29 Sep 2015

A Molecular Framework for Understanding DCIS

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0111

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

DCIS is proposed to be a precursor to invasive breast cancer. Improvements in early diagnosis have led to increased numbers of DCIS cases, however, we presently have no way to predict which DCIS lesions are at risk for progression to invasive cancer. We also lack the extensive molecular profiling necessary to place DCIS within the framework used to classify and guide treatment for invasive disease. We propose to take advantage of a unique set of specimens, comprising DCIS and early invasive disease, and using next-generation sequencing, understand the nature of DCIS and the events that determine and promote its progression. We have already developed the approaches necessary for obtaining profiles from the tumor and stromal compartments of DCIS, lesions from which only limited numbers of cells can be obtained. The availability of our datasets will transform the understanding of early disease and may ultimately alter the course of treatment for women with a diagnosis of DCIS.

15. SUBJECT TERMS

Breast cancer, DCIS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U					USAMRMC
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	7	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. Introduction

This project centers on creating a molecular framework of DCIS (ductal carcinoma in situ). DCIS is considered to be the precursor to Invasive Ductal Carcinoma (IDC), the most common form of breast cancer. IDC accounts for 80% of all breast cancers, predominantly affecting women aged 55 and older; however, at least a third of women with IDC are diagnosed before they reach 55.

Utilizing a unique bank of frozen mammary biopsies, containing samples with DCIS alone, and a combination of DCIS and IDC, we aim to profile both DCIS and related tissue components. It is our aim to sample the ~300 biopsies, and compare both by RNA seq, and whole genome amplification, DCIS lesions, within, and between patients, and see how these may be correlated with IDC lesions. We also intend to look for changes in the stroma between those patients that present with IDC and those that do not. This work aims to identify characteristics that may be suggestive of a patients' likelihood of progressing from DCIS to IDC, with the purpose of reducing the need for over treatment for this disease.

2. Approach

We first applied and received appropriate regulatory approval from the Duke SPORE tissue use committee, the Duke Cancer Center Protocol Review Committee, the Duke IRB, and the DoD. An MTA was established with the University of Cambridge.

We have created a DCIS Clinical Annotation database which was created and is being managed by Cedars-Sinai Medical Center in Los Angeles. A remote, web based case report from was also created to enable data abstraction for Duke medical records.

All Duke breast cancer research specimens were identified and prioritized based on pathologic diagnosis. Clinical data was abstracted from Duke clinical data by prioritizing pure DCIS, mixed DCIS and invasive BC samples, and invasive BC samples. Normal breast and atypical will be annotated if needed as controls.

Data was gathered from 1) electronic medical records at Duke University Medical Center and 2) extracted from DCIS patient charts (which hold the specific clinical annotation including patient outcome).

After identifying breast cancer research specimens with pathologic and clinical data consistent with DCIS and/or DCIS and invasive breast cancer, shipping to Dr. Greg Hannon for further processing was performed.

3. Key Research Accomplishments

Major goals of the project (as stated in SOW)

1. Regulatory approval and MTA (Duke/Cambridge)
2. Database creation and remote web based CRF created (Duke)
3. Sample collection/annotation, shipping to Cambridge (Duke)
4. Laser capture of frozen material (Duke pathologist working remotely or at CSHL/Cambridge)

5. Exome capture and DNA sequencing (CSHL/Cambridge)
6. RNAseq library construction (CSHL/Cambridge)
7. Analysis DNA data (CSHL/NYGC)
8. Analyze RNA differential expression (NYGC)
9. Analyze stroma compartments (CSHL/Cambridge)
10. Technical validation of potential markers (Cambridge)
11. Validate potential markers in FFPE cohort (Duke/Cambridge)
12. Validate in longitudinal cohort (Duke /Cambridge)
13. Nominate candidates for clinical validation (Duke /Cambridge)

Dr. Greg Hannon of Cancer Research UK Cambridge Institute will provide his research accomplishments via a separate report.

What was accomplished under these goals:

This year we have initiated goal 1.

Identification and clinical annotation of all of the DCIS and DCIS/IDC samples contained in the Duke SPORE tissue bank has begun.

Clinical Annotation: Identification of all of the cases of DCIS and DCIS/IDC from the Duke SPORE tissue bank, initiation of the clinical annotation of these selected cases in our current Breast Cancer Clinical Annotation Database. This database was created on the REDCap platform in collaboration with Cedars Sinai Medical Center (CSMC). This database adheres to CSMC Enterprise Information Services (EIS) research database security standards and contains no PHI.

To date we have annotated 108 cases of DCIS and DCIS/IDC contained in the SPORE tissue bank.

Pathologic and Clinical Annotation Database

- Housed on server at Cedar-Sinai Medical Center
  - No PHI
- Platform is REDCap (includes full functionality of REDCap)
- Accessible on the web via https (no VPN required)
- Database adheres to CSMC Enterprise Information Services (EIS) research database security standards
- Database consists of:
  - 9 Baseline forms (125 Clinical fields)
  - 4 Follow-up forms (65 Clinical fields)

2060 lesions that have sectioned on the LCM post annotation by the Duke breast pathologist Dr. Joe Geradts working remotely or on site at Cambridge. These include DCIS lesions, IDC, stroma adjacent to DCIS, stroma adjacent to IDC, atypia lesions, Stroma away from disease, normal ductal epithelium and immune infiltrates.

Dr. Greg Hannon of Cancer Research UK Cambridge Institute will provide his research accomplishments creating libraries and sequencing these samples via a separate report.

Opportunities for training and professional development

Nothing to report (not intended for training)

Results disseminated to communities of interest

Nothing to report

Plan for next reporting period

We plan to continue to clinically annotate the Duke frozen tissue breast bank. We will then extend our clinical annotation to the archival breast samples from Duke pathology, consisting of formaldehyde fixed paraffin embedded tissue after identifying these from the Duke pathology reports.

These samples will be identified and initial attempts to assemble them into a validation cohort for subsequent markers.

4. Reportable outcomes

Nothing to report

Actual or anticipated problems or delays and plans to resolve

The Duke pathologist is working either remotely or on site to perform the tissue microdissection. If this arrangement continues to prove effective, no changes will be made. However, more feasible alternatives, including the use of other pathologists, will be considered.

Changes in use or care of human subjects

Nothing to report

Products

Nothing to report

5. Participants & Other Collaborating Organizations

Individuals worked on the project

Name: **H. Kim Lyerly**
Project Role: Partnering PI – provide strategic insight into DCIS biology and clinical outcomes, and continues to engage thought leaders to support ongoing activities interrogating DCIS biology. Coordinates efforts to identify appropriate tumor samples for analysis from the Duke SPORE tissue bank, the clinical annotation of the samples in both the SPORE tissue bank, and the general Duke breast tissue bank, and administration of the the general tissue microdissection activities that will be directly supervised by Dr. Geradts and his team.
Nearest person month worked: 2.52 CM

Name: **Joseph Geradts**
Project Role: Co-investigator – microdissection of tissues and interpretation of stained tissue sections
Nearest person month worked: 1.2 CM

Name: **Katherine Kalinowski**
Project Role: Associate in Research – clinical data abstraction and annotation into secure DCIS clinical annotation database
Nearest person month worked: 4.8 CM

Name: **Amy Hobeika**
Project Role: Regulatory Administrator – ensures all necessary regulatory documentation is submitted and maintained
Nearest person month worked: 1.32 CM

Name: **Qing Cheng**
Project Role: Co-investigator – provide support for RNAseq analysis and bioinformatics coordination of genomics data with the clinical outcomes data with the clinical database
Nearest person month worked: 3.18 CM

Name: **Kimberly Egler**
Project Role: Research Project Manager – oversees administrative and financial activities
Nearest person month worked: 2.4 CM

Name: **Delila Serra**
Project Role: Laboratory Research Analyst – performs immunohistochemical assays on breast tumor tissue
Nearest person month worked: 6 CM

Name: **Karrie Comatas**
Project Role: Laboratory Research Analyst – performs immunohistochemical assays on breast tumor tissue
Nearest person month worked: 6 CM

Change in active support since last report

Nothing to report (this is the first reporting period)

Other organizations involved as partners

Cancer Research UK Cambridge Institute – collaboration to perform the RNA sequencing and the DNA sequencing and have laser microdissected 35 patients, as detailed in the grant application.

Cedars Sinai Medical Center (CSMC) – storage and management of secure DCIS database; as detailed in the grant application.

6. Conclusions

Annotation will continue and identification and annotation of the validation cohort from the Duke pathology archives will continue.